Perinatal Development of Conjugative Enzyme Systems

by George W. Lucier*

The problems and priorities involved in studying the role of conjugagive enzymes in developmental pharmacology are discussed and evaluated. The relative rates of UDP glucuronyltransfererase and β-glucuronidase were studied during perinatal development in hepatic and extrahepatic tissues to determine the net balance of glucuronidation or deglucuronidation at different developmental stages. In general, deglucuronidation predominated over glucuronidation in fetal tissues whereas the converse was evident in adults. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an extremely toxic contaminant of some organochlorine compounds, was shown to be a potent inducer of some hepatic and extrahepatic drug-metabolizing enzymes. TCDD, administered during gestation, induced the postnatal activities of p-nitrophenol glucuronyltransferase and benzpyrene hydroxylase in rats. Foster mother experiments revealed that the postnatal induction was caused primarily by newborn exposure to TCDD in the mother's milk. Tissue distribution experiments with TCDD-¹⁴C confirmed these findings. Although TCDD induced nonsteroid glucuronidation, no significant effects were evident on the postnatal development of steroid glucuronidation. The synthetic estrogen diethylstilbestrol (DES) is metabolized primarily by glucuronidation. The postnatal development of DES glucuronidation, like the steroid pathway, was not affected by gestational TCDD treatment. The fetal distribution of DES and DES-glucuronide, at different stages of development, correlated well with the perinatal development of steroid glucuronyltransferase activity.

Introduction

Many of the inherent problems in studying perinatal drug metabolism have been described previously (1), and in general these apply to development aspects of conjugative enzymes as well.

On simplification, and depending on chemical structure, the drug biotransformation process may be separated into two parts: first an oxidative reaction, such as hydroxylation, results in the formation of a free hydroxyl group; this is then rapidly conjugated with glucuronic acid, sulfate, or another conjugate (2 a). This series of reactions renders the molecule more polar and generally more excretable. Although the conjugative enzymes do not appear to be as complex as the mfo systems, there are many complicating

factors. For example, deconjugative reactions such as β-glucuronidase (βG) and aryl sulfatase perform the opposite reaction to form a less polar compound; liberating the free aglycone in the case of βG (3). Like the mixed function oxidase (mfo) enzymes, many of the conjugative processes also play important roles in steroid metabolism as well as drug metabolism (4). Glucuronides are often considered end products of metabolism, whereas sulfates play an integral role in steroidogenesis (5), an area of obvious concern to the developmental toxicologist.

Some of the conjugative enzymes, most notable uridinediphosphoglucuronyltransferase, (UDP glucuronyltransferase, UDPGT), are microsomal and inducible by many of the same compounds that induce mfo enzymes, such as TCDD and phenobarbital (6-9). However, these enzymes are not P-450-dependent and some, such as the sulfotransferases, are located in the soluble fraction of mammalian cells (10).

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The first section of this paper will outline important objectives in studying the perinatal development of conjugative enzymes and this will be followed by recent research data related to these objectives. Most of the examples will involve UDPGT because of the available literature in developing systems, although other enzyme systems are also important. The sulfotransferases have been given relatively little attention because of the difficulty in obtaining sufficient quantities of phosphoadenosine phosphosulfate, the cofactor in sulfate transfer. This is now becoming commercially available, and sulfation will become a more easily studied process. N-Acetyltransferase (NAT) functions in metabolism of some endogenous amines such as serotonin and the deactivation of many drugs (11). Glycine conjugation is involved in the metabolism of some carbamate pesticides and functions in the heme biosynthetic pathway (2b).

Perinatal Development of Conjugative Enzyme Systems: Objectives

Characterize the Perinatal Development Patterns of Conjugative Enzyme Systems in Experimental Animals

Like other biochemical processes, the activity of conjugative enzymes can vary tremendously depending on age, species, strain, time of day, and with exposure to other xenoboitics. In regards to mixed function oxidase activity, human fetal microsomes have significant activity, but experimental animals, at least noninduced animals, have low activities (12,13). In contrast, UDPGT has low activity in human fetal and kidney microsomes, whereas activity is quite high in the livers of the late fetuses of many experimental animals (8,14). These data constitute serious drawbacks in attempts to extrapolate data from experimental animals to the human condition. The low activity of most enzymes in fetal tissues often makes their measurement difficult. However the recent development of sensitive radiochemical procedures should help alleviate this problem (15,16).

Investigate Developmental Patterns of Steroid Conjugative Enzymes as Well as Nonsteroid Pathways

As mentioned previously, the conjugative pathways play an important role in steroid metabolism as well as drug metabolism; especially sulfation and glucuronidation. There is considerable evidence supporting the idea that a different set of UDPGT conjugates steroids than nonsteroids (8,17-19). Although the evidence is not entirely conclusive, it is reasonable to assume that the developmental patterns, tissue distributions, and responses to inducing agents might be different for steroid conjugations compared to nonsteroid pathways.

Determine Developmental Patterns in Extrahepatic as Well as Hepatic Tissues

In recent years, increasing attention has been placed on the role of extrahepatic drug-metabolizing enzymes in fetuses as well as adults (20,21). The fetus has to be considered differently from adults, since foreign chemicals reach the fetus via the placenta whereas several routes of exposure are possible after birth. Also, fetal biotransformation capabilities differ markedly from those of mature animals (22). Of course, the amount of parent compound or metabolites entering the fetus will vary depending on the mode of maternal exposure. It is important to determine the onset of conjugative activity in specific fetal tissues. This information should provide insight into how the fetus independently handles foreign chemicals at different developmental stages. We should also attempt to correlate these factors with susceptibility to transplacental toxicants.

Quantify the Balance between Conjugative and Deconjugative Processes in Different Tissues at Different Developmental Stages

The net conjugation and fate of conjugates in a particular tissue depends on a number of factors, including deconjugation, other metabolisms, transport, and binding to specific and nonspecific sites. The ability of a xenobiotic to elicit a physiological response depends on the binding properties of that chemical and how subsequent metabolism affects binding properties. Therefore, there is a strong rationale to study the ontogeny of drug-receptor interactions as well as the ontogeny of drug metabolism. Bearing on this problem is the potential for xenobiotics and their metabolites to interfere with normal hormone-receptor interactions.

The net conjugation in vitro can be determined by measuring separately, conjugation and deconjugation. However, this approach is fraught with many problems, and it is difficult to extrapolate to the in vivo condition. There are several reasons for this. First, the pH optima for conju-

gative reactions *in vitro* are often quite different than for deconjugation reactions. For example, βG is most active at pH 4.5 (*3*) and UDPGT at pH 7.5 (*7*), with very little cross activity at the respective pH values. Of course, the possibility exists that there are microspheres of low pH *in vivo* allowing for βG activity. Another complicating factor involves the necessity to activate UDPGT by adding microsomal membrane perturbing agents to *in vitro* tissue preparations (*23-26*). The *in vitro* incubation conditions that most adequately reflect *in vivo* activities is a question which has not been adequately resolved. In addition, *in vitro* activation of fetal tissues might differ qualitatively or quantitatively from that of adult tissues, depending on the morphological localization of enzyme on the endoplasmic reticulum.

Conjugation and deconjugation reactions are of particular interest in the placenta, since the fetal-maternal transfer of some steroids is regulated by the net deconjugation rates (*5*). This is particularly true for the steroid sulfates.

Determine Alteration of Perinatal Enzyme Development by Gestational Exposures to Environmental Chemicals

It is well known that some conjugative enzymes are induced by certain xenobiotics (*6-9*). We feel that is is important to determine how the perinatal development of steroid and nonsteroid biotransformation activities might be changed by gestational or newborn exposure to environmental contaminants. We should also determine which tissues are most sensitive to perinatal induction or suppression.

Correlate Developmental Changes in Enzyme Activity with Changes in Toxicity and Fetal Pharmacology of Foreign Compounds

Scientists working in the area of perinatal pharmacology should attempt to correlate developmental enzyme fluctuations with the pharmacology of foreign compounds. We should also use pharmacokinetics as a tool to ascertain periods of fetal susceptibility to transplacental toxicology.

Do Changes in Developmental Patterns of Conjugative Enzymes Provide Reliable Indicators of Developmental and Reproductive Toxicity?

An area of recent concern to the NIEHS involves the identification of biochemical indicators that precede or accompany developmental toxicity. There is some evidence that the developmental pattern of conjugative enzyme pathways could relfect impending toxicity. This area is covered in detail in a separate article (*27*).

What are the Factors that Regulate Onset of Conjugative Activity in the Fetus or Newborn?

This objective is of great importance to developmental toxicologists in relation to the factors that could delay or prematurely initiate onset of enzyme activity. Dutton's group in England has done considerable and elegant work on this subject (*19*). They have used chick embryo liver cultures to study the developmental induction of glucuronyltransferase activity. Enzyme activity increases markedly in culture and actually exceeds that found normally in adult liver. Figure 1 illustrates the glucuronidation of a phenolic compound catalyzed by UDPGT. This process requires UDPGA as the cofactor and glucuronic acid donor. UDPGA synthesis is catalyzed by an NAD+-dependent UDPG dehydrogenase and is degraded by a nucleotide pyrophosphatase. However fetal UDPGA condentration is not the primary factor in regulating the onset of glucuronidation activity. The glucuronide product can also be degraded by βG, and high levels of this hydrolytic activity might be responsible for low fetal glucuronidation rates. However, this was also shown not to be the case, since βG inhibitors did not alter glucuronidation rates, and βG had only negligible activity at the pH used to measure glucuronidation. THe conclusion was that developmental onset of glucuronidation appears to be regulated by increased amounts of UDPGT (*19,28*). This idea was further reinforced by the ability of protein synthesis inhibitors to prevent the developmental onset of activity (*29*). However, the situation is complicated by the fact that
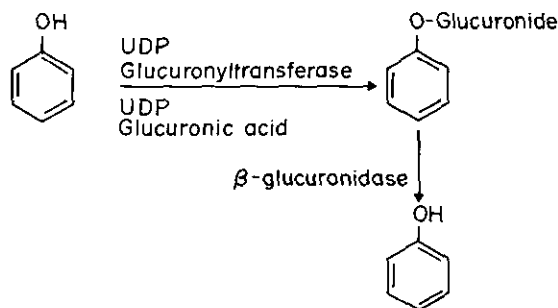

FIGURE 1. Schematic representation of UDPGT and βG activity.

yolk added to the tissue culture delays the development of glucuronyltransferase (19).

Perinatal Development of Glucuronyltransferase and β-Glucuronidase

The perinatal development of microsomal β-glucuronidase (βG) and UDP glucuronyltransferase (UDPGT) was investigated in rabbits, rats, and guinea pigs. Substrates used in these studies were p-nitrophenol (PNP) and p-nitrophenyl β-D-glucuronide. Results for guinea pig tissues are illustrated on Figures 2-6. βG is located in both microsomal and lysosomal fractions. The data illustrated in Figures 2-6 represent the developmental patterns for βG only in microsomes, since the patterns were similar for both microsomes and lysosomes. In liver, glucuronidation was first evident at day 38 of gestation, and activity gradually increased. The increase was dramatic near birth, so that for the first week after birth enzyme activity exceeded adult levels. βG activity developed prior to UDPGT in liver and deconjugation exceeded conjugation until day 54 of gestation. Glucuronidation predominated at all subsequent developmental stages; the difference reaching a maximum of sevenfold by one week after birth and threefold in adults. In general, rats exhibited similar developmental patterns as guinea pigs but rabbits were different, in that no developmental peak in UDPGT activity about the time of birth was evident. Guinea pig lung exhibited a different developmental pattern than liver. UDPGT was not detectable until just after birth, and adult activities were low; being approximately 1 nmole/min/mg protein, compared to approximately 50 in liver. βG was active in fetal lung and activities increased sharply about the time of birth. Deglucuronidation exceeded glucuronidation at all developmental stages tested based on specific activities. The difference was approximately fourfold in adults.

Unusual developmental patterns were observed in the intestine with regard to βG. Fetal activities were extremely high: approximately 50 nmole/min/mg microsomal protein. βG activities decreased sharply after birth, reaching adult levels of approximately 5 nm/min/mg by 21 days after birth. Similar high levels of fetal and newborn intestinal βG were observed in rabbits and rats. The importance of βG in fetal and newborn intestine is not clear. Since neoplastic tissues
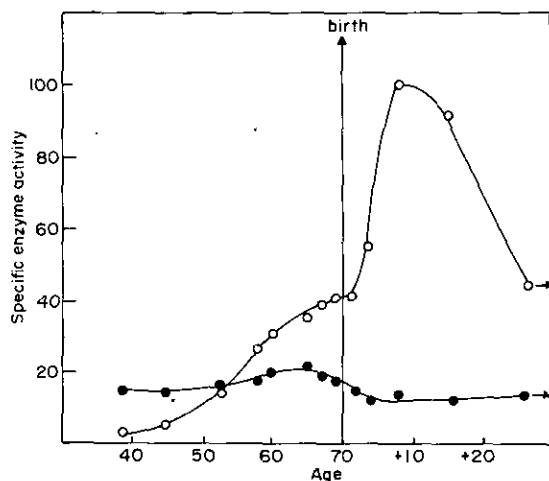

FIGURE 2. Perinatal development of (O) UDPGT and (•) βG in guinea pig liver. Each litter was pooled and each point represents the mean of three separate litters.
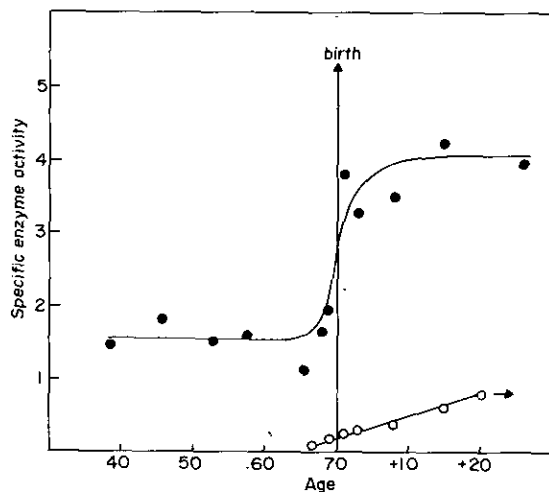

FIGURE 3. Perinatal development of (O) UDPGT and (•) βG in guinea pig lung. Each litter was pooled and each point represents the mean of three separate litters.

often exhibit enzyme profiles similar to fetal tissues (30) it would be of interest to determine if βG isozymes are good biochemical markers for intestinal carcinogenesis. Intestinal UDPGT, like the lung enzyme, did not develop until birth and gradually attained adult levels by 3 weeks after birth. Deglucuronidation exceeded glucruonidation at all developmental stages; the difference being 1.5-fold in adults and greater than 500-fold in fetal intestine.

Kidney βG remained relatively constant throughout development; approximately 3 nmole/min/mg protein. In contrast, UDPGT was
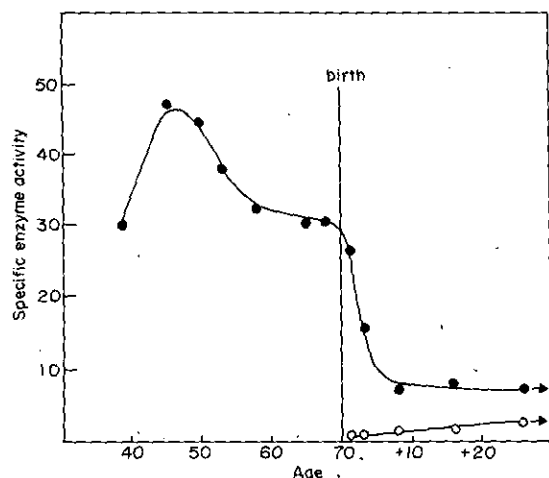
FIGURE 4. Perinatal development of (O) UDPGT and (●) βG in guinea pig intestine. Each litter was pooled and each point represents the mean of three separate litters.

FIGURE 5. Perinatal development of (O) UDPGT and (●) βG in guinea pig kidney. Each litter was pooled and each point represents the mean of three separate litters.
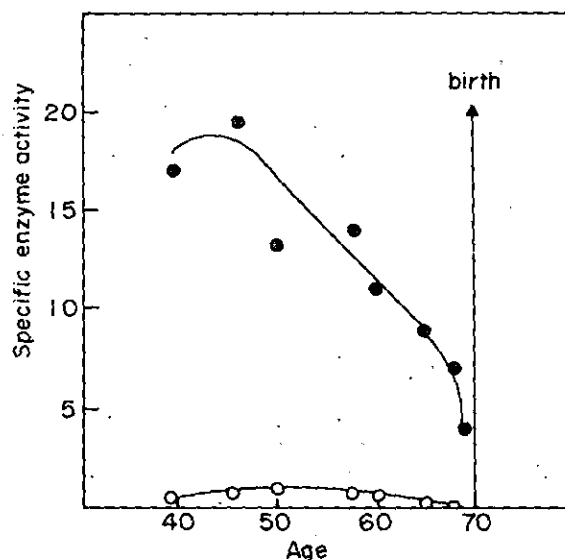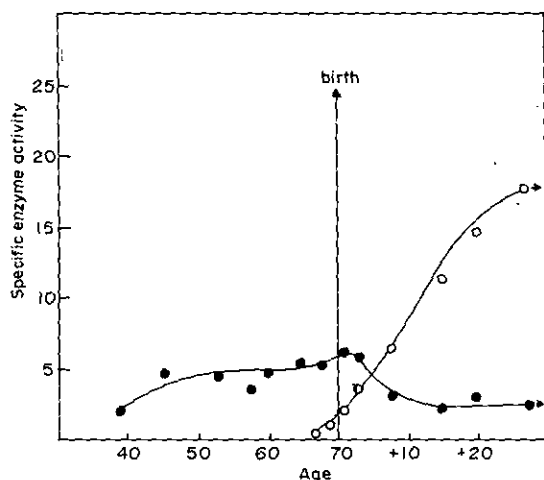

FIGURE 6. Fetal development of (O) UDPGT and (●) βG in guinea pig placenta. Each litter was pooled and each point represents the mean of three separate litters.

not detectable until 2 days prior to birth and increased rapidly until 2 weeks after birth, when adult levels were attained. Deglucuronidation exceeded glucuronidation at all fetal stages and for the first week after birth. UDPGT specific activity was sevenfold greater than βG in adult animals. Placental βG levels were high throughout gestation although activities decreased near term. The highest βG specific activity observed was 23 nmole/min/mg protein at day 50 of gestation. Placental UDPGT activities were low during gestation and nondetectable near term. In general, deglucuronidation exceeded glucuronidation by approximately 10-fold.

These results clearly illustrate the large variations in relative activities of UDPGT and βG, depending on tissue and developmental stage. Of particular note was the general predominance of βG in fetal tissues. Although these types of studies are complicated by many factors, indications are that net glucuronidation rates will be generally low in fetal tissues. Since glucuronidation is considered a detoxication process, the relative activities may be partially responsible for the fetal susceptbility to some foreign chemicals. Of course, the role of UDPGT and βG in the activation of carcinogenic amines (31) also emphasizes the need to quantify both these enzymes in specific tissues. Fetal clearance of glucuronides should be relatively slow compared to maternal excretion due to the lack of placental permeability to conjugates although the retrograde transfer of chemicals is poorly understood. For example, following IV administration of ³H-diethylstilbestrol (DES) to pregnant mice, DES-glucuronide concentrations in fetal plasma were twice that of maternal plasma (32).

Perinatal Induction of Glucuronyltransferase

After establishing the perinatal developmental patterns, the next step was to determine if the patterns could be altered. The chemical that we selected, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is an extremely toxic contaminant of

some organochlorine compounds, most notably the herbicide 2,4,5-trichlorophenoxyacetic acid (33). The oral LD₅₀ ranges from 1 μg/kg in guinea pigs to 100 μg/kg in rats. We have also shown that TCDD is an extremely potent and persistent inducer of some microsomal enzymes in rats; approximately 50000 times more potent than the classic including agent phenobarbital (Table 1, Fig. 7) (6). Induction is apparently caused by an increase in enzyme systheses as evidenced by kinetic data, UDPGT solubilization and partial purification studies, and the prevention of induction by actinomycin D (7).

TCDD, after administration to pregnant rats on day 5 of gestation, did not affect fetal glucuronidation rates of PNP (Fig. 8). However, a marked postnatal inductive effect was observed. The increase was twofold 1 week after birth, and maximum induction (eightfold) occurred 3 weeks after birth. UDPGT activity 52 days after birth was still increased twofold. The developmental patterns were similar when pregnant rats were administered TCDD on the days 5, 10, or 16 of gestation. TCDD effects were also similar when PNP, 1-naphthol, or methylumbelliferone were used as substrates. Postnatal induction was evident in extrahepatic as well as hepatic tissues (Table 2) and occurred for hydroxylation reactions (8).

The postnatal inductive effect must have resulted either from newborn exposure to TCDD in milk or to the activation of an inducing mechanism about the time of birth. Data from cross-

Table 1. Change in activities of female rat liver microsomal enzymes following a single oral dose of TCDD.[a]

Enzyme	Relative change from control values (100)[b]			
	TCDD, 0.05 μg/kg	TCDD, 0.2 μg/kg	TCDD, 1.0 μg/kg	TCDD, 5.0 μg/kg
Cytochrome P-450	—	126[c]	153[c]	196[c]
Benzphetamine de-methylation	—	115	112	118
Benzpyrene hydroxylation	351[c]	783[c]	1225[c]	1403[c]
Glucuronyltransferase	142[c]	257[c]	506[c]	687[c]

[a] Rats were killed 3 days after TCDD treatment at various dose levels. Each value derived from four animals.
[b] Data calculated on the basis of specific activities.
[c] Significantly different from controls at least at $p < 0.05$.
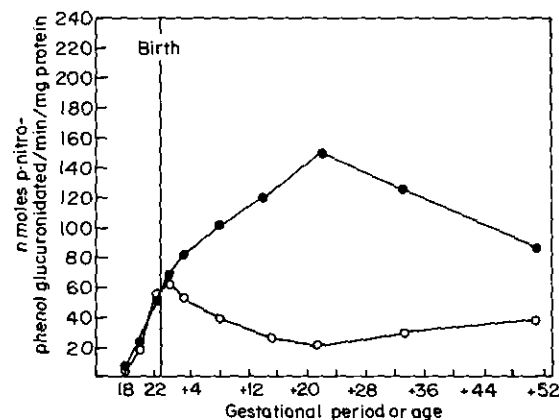
FIGURE 8. Effects of oral TCDD treatment (3 μg/kg) of pregnant rats on the development of hepatic fetal and newborn PNP glucuronidation: (O) control; (•) TCDD. Each litter was pooled and each point represents the average of three separate litters.
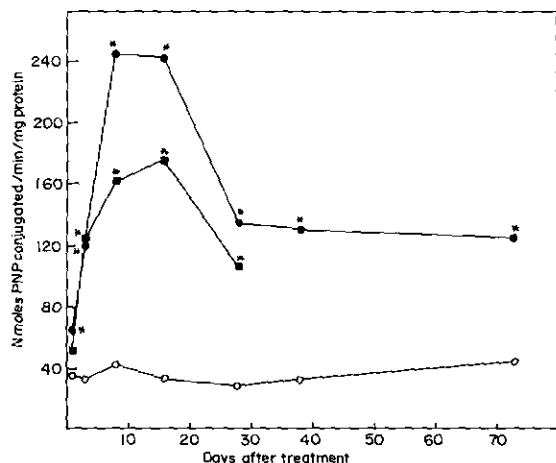
Table 2. Postnatal induction of hepatic and extrahepatic glucuronyltransferase by TCDD in rats.[a]

Tissue	PNP conjugated, nmole/min/mg protein		Induction factor
	Control	TCDD	
Liver	27.3	159.4	5.8
Lung	0.3	2.9	9.7
Kidney	8.4	87.7	10.4
Intestine	1.2	15.5	12.9
Testes	6.6	7.0	1.1

[a] Rats treated (3 μg/kg) on day 15 of gestation and sacrificed 8 days after birth.

FIGURE 7. Time course effects of a single oral dose of TCDD on male rat liver UDPGT: (O) controls; (□) 5 μg TCDD/kg; (•) 25 μg TCDD/kg. N=3 rats. An asterisk indicates that values are significantly different from controls at least at $p < 0.05$.

fostering experiments presented in Table 3 indicate that exposure of newborns to milk containing TCDD was the primary factor. Secretion of TCDD in milk appears to be an extremely efficient excretory system, since maternal induction levels decreased sharply postpartum after gastational TCDD treatment (Fig. 9). If pups were removed from TCDD-treated mothers immediately after birth, preventing suckling, the maternal induction decay curve was less rapid. Induction levels in fetal and newborn tissues correlated well with tissue distribution experiments following treatment of pregnant rats with ^{14}C-TCDD on the day 11 of gestation (J. Moore, NIEHS, unpublished observations). One day prior to birth, each fetus had received a dose of 0.3 µg/kg, whereas 14 days after birth the dose was 1.8 µg/kg. Each fetus received approximately 1.0 ng TCDD by the day prior to birth, compared to 40 ng 14 days after birth.

FIGURE 9. Effects of oral TCDD treatment (3 µg/kg) of pregnant rats on the time course of maternal hepatic UDPGT activities: (O) control; (•) TCDD. Each point represents three animals.

Table 3. Postnatal induction of hepatic microsomal enzymes by TCDD treatment (3 µg/kg) of pregnant rats following cross-fostering of control and TCDD-treated groups.[a]

Animals[b]	PNP glucuronidation nmole/min/mg microsomal protein	Benzpyrene hydroxylation, nmole 3-hydroxybenzpyrene/min/mg microsomal protein
Group 1	18.6 ± 1.5	0.227 ±0.016
Group 2	99.5 ± 6.4[c]	1.115 ±0.083[c]
Group 3	100.4 ± 8.9[c]	1.143 ±0.091[c]
Group 4	33.3 ± 2.4[c]	0.604 ±0.073[c]
Group 5	95.6 ±12.3[c]	0.604 ±0.073[c]

[a] Pregnant rats were treated on the day 5 of gestation, and the newborns were sacrificed 8 days after birth.

[b] Group 1, control mothers nursing control pups; group 2, TCDD-treated mothers nursing TCDD-treated pups; group 3, TCDD-treated mothers nursing control pups; group 4, control mothers nursing prenatally TCDD-treated pups; group 5, mother rats administered TCDD immediately after giving birth.

[c] Significantly different from group 1, $p<0.01$.

UDPGT Multiplicity

Considerable research has been documented recently to demonstrate the enzyme multiplicity of glucuronyltransferase (7,8 17-19). Research in our laboratories has provided evidence that perinatal developmental patterns of steroid glucuronidations differ significantly from nonsteroid pathways (8). Steroid substrates used in these studies were testosterone, β-estradiol, and estrone.
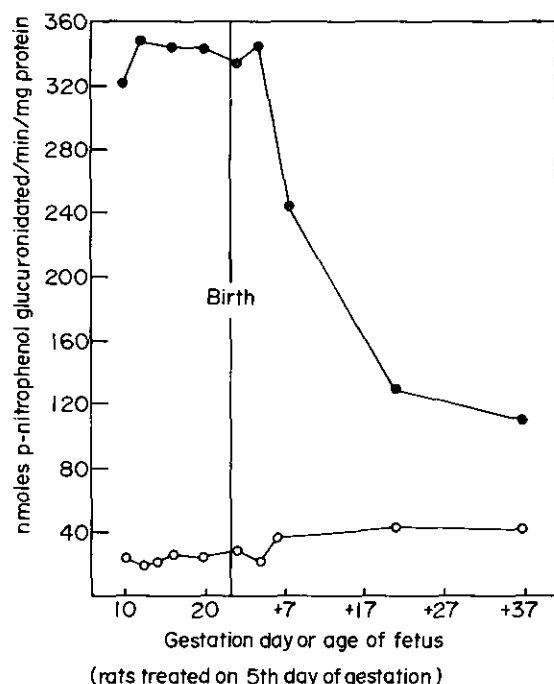
The normal developmental patterns of steroid glucuronidations in control and TCDD- treated rats are illustrated in Figure 10. The perinatal patterns differed from nonsteroid glucuronidations in two ways: (1) there was no developmental peak in enzyme activity at birth such that glucuronidation rates at birth exceeded adult activities; and (2) TCDD did not cause postnatal induction of steroid glucuronidations. β-Estradiol has two sites for glucuronidation: at the 3-OH and 17-OH positions. The perinatal patterns at both sites were similar, and neither was TCDD-inducible. A third differentiating factor involves tissue distributions of steroid and nonsteroid UDPGT. Nonsteroid glucuronidation enzymes have relatively high activities in male and female reproductive tract tissue, whereas steroid UDPGT is low or nondetectable (34). A summary of steroid and nonsteroid substrates is presented in Table 4. Phenolphthalein, which has estrogenic activity, was glucuronidated as a steroid.

The synthetic estrogen, diethylstilbestrol (DES), has potent steroidal properties as well as being a foreign chemical. DES has been used as a gynecological treatment and cattle food additive and has recently received considerable attention
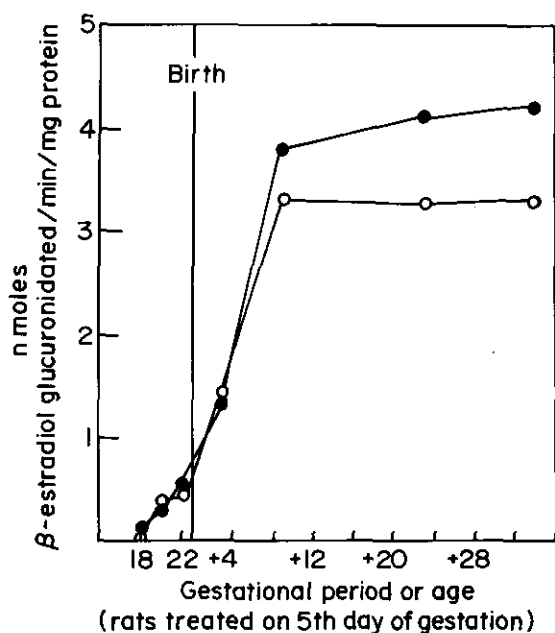
FIGURE 10. Effects of oral TCDD treatment (3 µg/kg) of pregnant rats on the development of hepatic fetal and newborn β-estradiol glucuronidation: (○) control; (●) TCDD. Each litter was pooled and each point represents the average of three separate litters.
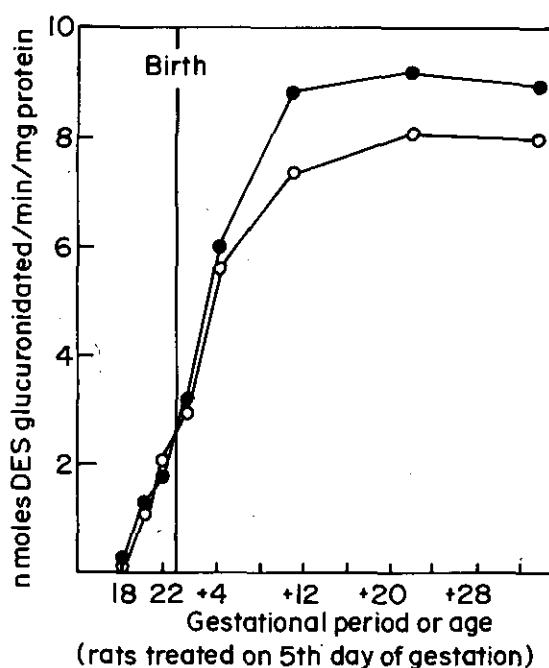

FIGURE 11. Effects of oral TCDD treatment (3 µg/kg) of pregnant rates on the development of hepatic fetal and newborn DES glucuronidation: (○) control; (●) TCDD. Each litter was pooled and each point represents the average of three separate litters.

Table 4. Summary of glucuronidation characteristics of some steroid and nonsteroid substrates.[a]

Substrate	Develop-mental peak	TCDD induction	Reproductive tract activity
p-Nitrophenol	+ + +	+ + +	+ + +
1-Naphthol	+ + +	+ + +	+ + +
4-Methylumbel-liferone	+ + +	+ + +	+ + +
Testosterone	—	—	—
Estradiol	—	+	—
Estrone	—	—	—
Diethylstilbestrol	—	+	+
Phenolphthalein	—	—	—

[a] +++ denotes pronounced effect; + indicates slight effect; — indicates lack of effect or activity.

because of its implication as a transplacental carcinogen (35,36). It also appears that DES causes deficits in reproductive tract function and altered morphology in offspring following gestational exposures. Our studies were directed initially to determine whether DES is glucuronidated as a steroid or nonsteroid. The major metabolic pathway for DES is glucuronidation (37), although some nonpolar metabolites have been identified (38). The perinatal development in rats is illustrated on Figure 11; the data clearly indicate that DES is glucuronidated as a steroid.

Influence of UDPGT on Perinatal Pharmacology

Perinatal developmental patterns, alone, have limited value. Therefore, we performed experiments to determine if the perinatal developmental pattern of DES UDPGT correlated with fetal levels of DES-glucuronide following ³H-DES administration to pregnant mice at varying gestational ages. Mice were used in these studies rather than rats because of the availability of a well-defined mouse model for DES-induced transplacental carcinogenesis (36). It is unlikely that significant quantities of DES-glucuronide formed in the maternal compartment would cross the placenta. Therefore it is reasonable to assume that DES-glucuronide detected in the fetus was formed in the fetal compartment. Results (Table 5) indicate that during the earlier gestational ages, when DES UDPGT activities are low or undetectable, only small amounts of ³H-DES glucuronide were present in fetal tissues. As hepatic DES UDPGT activities increased during development, fetal levels of ³H-DES glucuronide increased concommitantly. It is also interesting to note that, although ³H-DES glucuronide concentrations increased with gestational age, fetal

Day of gestation	DES, ng/g fetus [a,b]	Conjugates, DES ng/g fetus [a,b]	UDPGT activity in fetal liver, nmde/DES glucuronide/min/ mg microsomal protein
11	1.10 ± 0.05	0.10 ± 0.02	—
12	1.13 ± 0.07	0.21 ± 0.02	—
14	1.47 ± 0.10	0.55 ± 0.07	$0.17 \pm .004$
16	1.20 ± 0.10	1.74 ± 0.20	4.80 ± 0.24

[a] Concentration of DES and its conjugates in whole fetuses 30 min after IV administration of tritiated DES (30 µg/kg). Each value represents mean $\pm$ S.E. of observations in 3 mice.

[b] Each value represents mean $\pm$ S.E. of observations in 4 mice. Fetal livers from the same litter were pooled.

concentrations of DES remained relatively constant. Therefore, there were significantly greater fetal concentrations of radioactivity at the later stages compared to the earlier. This finding might have toxicological significance, in that DES-glucuronide should not be readily cleared from the fetal compartment and DES could be liberated from the fetal DES-glucuronide pool by βG. DES was identified by Florisil chromatography and three thin-layer systems. Approximately 5% of the recovered radioactivity resided in unidentified nonpolar methabolites (*32*). In a recent report (*38*), several nonpolar metabolites of DES were identified in mammals, including dienstrol, hydroxyl, and methylated derivatives. It is possible that some of the unidentified compounds in mice (*32*) could be these metabolites. Metzler et al. (*38*) postulate that the dienstrol metabolites might be formed through an epoxide intermediate. It is concluded that the perinatal development of UDPGT plays an important role in the pharmacology and perhaps toxicity of many foreign chemicals. This enzyme system appears also to function in the metabolic regulation of steroid hormones during development and is sensitive to chemical insult in newborn as well as adult animals.

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